PTO 1542
(4 85)

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

RUSH 87245

ONLINE SEARCH REQUEST FORM

****

USER Irene Marx SERIAL NUMBER 09/622 385

ART UNIT 1651 PHONE 308-1922 DATE 2/21/03

Please give a detailed statement of requirements. Describe as specifically as possible the subject matter to be searched. Define any terms that may have special meaning. Give examples or relevant citations, authors, or keywords, if known.

You may include a copy of the broadest and or relevant claim(s).

Please search inventors

reaction to make I from II

with Escherichia or E. coli

transformed with gene for carbonyl reduction

a enzyme therefrom

E. coli JM109
HB101
DH5

plasmids pKAR
pKKGDH

Point of Contact:
Susan Hanley
Technical Info. Specialist
CM1 6B05 Tel: 305-4053

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=> D HIS

     (FILE 'HOME' ENTERED AT 15:46:58 ON 24 FEB 2003)

     FILE 'HCAPLUS' ENTERED AT 15:47:12 ON 24 FEB 2003
L1           402 S PETERSEN M?/AU
L2            14 S BIRCH O?/AU
L3          4273 S SHIMIZU S?/AU
L4             0 S KJENER A?/AU
L5             3 S HISCHIER M?/AU
L6             1 S THONI S?/AU
L7          4689 S L1-6
L8          7931 S ?HYDROXYBUTYRIC?
L9            10 S L7 AND L8
L10            9 S L9 NOT  COTTON/TI
                 SELECT RN L10 1-9

     FILE 'REGISTRY' ENTERED AT 15:50:14 ON 24 FEB 2003
L11           24 S E1-24

     FILE 'HCAPLUS' ENTERED AT 15:50:22 ON 24 FEB 2003
L12            9 S L10 AND L11

     FILE 'LREGISTRY' ENTERED AT 15:52:12 ON 24 FEB 2003
L13              STR

     FILE 'REGISTRY' ENTERED AT 15:54:32 ON 24 FEB 2003
L14            9 S L13
L15          144 S L13 FUL
                 SAVE L15 TEMP MAR385P/A
L16              STR L13
L17          109 S L16 SSS FUL SUB=L15
                 SAVE L17 TEMP MAR385KET/A
L18           35 S L15 NOT L17

     FILE 'HCAPLUS' ENTERED AT 15:59:27 ON 24 FEB 2003
L19          578 S L17
L20          489 S L19(L)RCT/RL
L21           98 S L18
L22           61 S L21(L)PREP/RL
L23           32 S L20 AND L22
L24           34 S L19 AND L22
L25           34 S L23-24
                 E ESCHERICHIA COLI+ALL/CT
L26       120959 S ESCHERICHIA COLI+PFT,NT/CT
L27            1 S L25 AND L26
L28            0 S L27 NOT L9
L29            1 S L25 AND ESCHERICH?
L30            1 S L25 AND COLI
L31            0 S L29-30 NOT L9
L32            3 S L25 AND (MICROORG? OR ENZYM? OR BIOTRANS?)
L33            2 S L32 NOT L9
L34            4 S L25 AND (CELL OR CELL-FREE OR MICROB?)
L35            2 S L34 NOT L32
                 E KIENER A/AU
L36           48 S E50-52
L37            1 S L8 AND L36
L38            0 S L37 NOT L9
L39           60 S (L7 OR L36) AND L26
L40           19 S L39 AND REDUC?
```

inv. name misspelled

Inventor search (see L41 also)

24 cpds in L10 cites

9 citations w/ 24 cpds displayed

144 cpds in full file search

109 diketo cpds

35 β hydroxy keto cpds

diketo

diketo as a reactant

β hydroxyketo

β hydroxyketo product

34 cites w/ both RCT & product

CT = controlled terminology

only 1 cite w/ E coli ✡ ✡

this is applicant's work

✡ ✡

2 cites

2 cites

these are the only other enzymatic (Bakers yeast/plant enz. reductions)

Appl. name is misspelled

E. coli

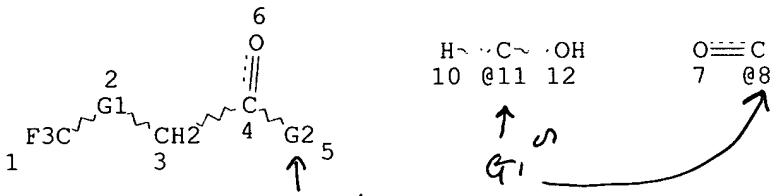
39

L41 18 S L40 NOT L9 18 cites related to Applicants & research w/ E. coli

search for diketo reactant

=> D QUE L19 parent STR

L13 STR



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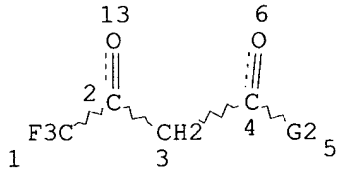
DEFAULT ECLEVEL IS LIMITED

GRAPH ATTRIBUTES:
RING(S) ARE ISOLATED OR EMBEDDED
NUMBER OF NODES IS 11

STEREO ATTRIBUTES: NONE
L15 144 SEA FILE=REGISTRY SSS FUL L13
L16 STR Subset search



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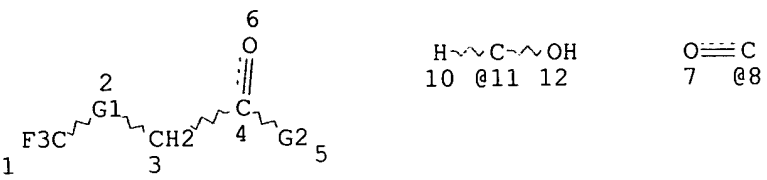
STEREO ATTRIBUTES: NONE
L17 109 SEA FILE=REGISTRY SUB=L15 SSS FUL L16 109 cpds
L19 578 SEA FILE=HCAPLUS ABB=ON PLU=ON L17 578 cites

search for β-hydroxy ketone product

=> D QUE L21
L13 STR parent STR



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VAR G2=N/O/S
NODE ATTRIBUTES:
DEFAULT MLEVEL IS ATOM
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STEREO ATTRIBUTES: NONE
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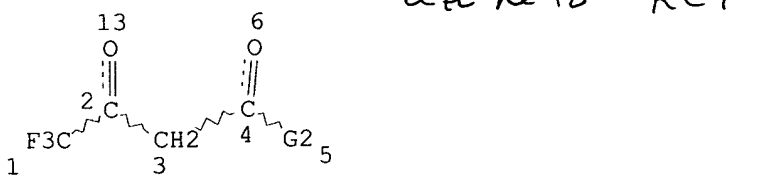
L16 STR diketo RCT STR



VAR G2=N/O/S
NODE ATTRIBUTES:
DEFAULT MLEVEL IS ATOM
DEFAULT ECLEVEL IS LIMITED

GRAPH ATTRIBUTES:
RING(S) ARE ISOLATED OR EMBEDDED
NUMBER OF NODES IS 7

STEREO ATTRIBUTES: NONE
L17 109 SEA FILE=REGISTRY SUB=L15 SSS FUL L16 ← reactant
L18 35 SEA FILE=REGISTRY ABB=ON PLU=ON L15 NOT L17 product cpd (35)
L21 98 SEA FILE=HCAPLUS ABB=ON PLU=ON L18

98 cites for 35 cpds

Inv. search

MARX 09/622,385

=> d ibib abs hitstr ind 1

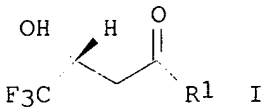
L12 ANSWER 1 OF 9 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1999:549386 HCAPLUS
DOCUMENT NUMBER: 131:183941
TITLE: Biotransformation for producing trifluoro-3(R)-**hydroxybutyric** acid derivatives using genetically engineered E.coli
INVENTOR(S): **Petersen, Michael; Birch, Olwen; Shimizu, Sakayu**; Kiener, Andreas; **Hischier, Marie-Luise; Thoni, Susanne**
PATENT ASSIGNEE(S): Lonza A.-G., Switz.
SOURCE: PCT Int. Appl., 27 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

priority Doc - only applicant does this rxn w/ E coli

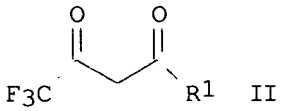
PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9942590	A1	19990826	WO 1999-EP1017	19990218
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
CA 2311649	AA	19990826	CA 1999-2311649	19990218
AU 9929265	A1	19990906	AU 1999-29265	19990218
EP 1054974	A1	20001129	EP 1999-910229	19990218
R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, NL, SE, PT, IE, FI				
JP 2002504337	T2	20020212	JP 2000-532530	19990218

PRIORITY APPLN. INFO.: CH 1998-388 A 19980218
WO 1999-EP1017 W 19990218

OTHER SOURCE(S): MARPAT 131:183941
GI



I



II

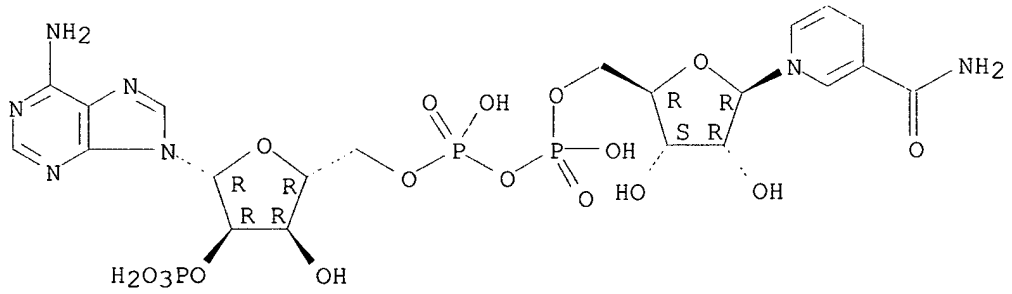
AB The invention relates to a biotechnol. method for producing trifluoro-3(R)-**hydroxybutyric** acid derivs. of the general formula (I), where R1 represents -OR2, where R2 is hydrogen, C1-10 alkyl, C1-10 alkenyl, C3-8 cycloalkyl, aryl, alkoxyalkyl or alkoxyalkoxyalkyl; -NR3R4, where R3 and R4 are the same or different and represent hydrogen, C1-10 alkyl, C1-10 alkenyl, C3-8 cycloalkyl or aryl; or -SR5, where R5 represents hydrogen, C1-10 alkyl, C1-10 alkenyl, aryl or C3-8 cycloalkyl, based on a trifluoroacetoacetic acid deriv. of the general formula (II), where R1 has the meaning given above, by means of microorganisms which are able to reduce a carbonyl function or by means of a cell-free enzyme ext. of said microorganisms. Biotransformation is performed using Escherichia Coli strains JM109 or HB101 contg. the plasmids pKAR and pKKGDH coding for NADPH dependent aldehyde reductase and the NADPH-generating glucose dehydrogenase. The formed products are pharmaceutical intermediates, e.g. for befloxatone. Fermn. is performed in one phase or two phase systems at 5-60.degree.C and pH 5-10. Thus 4,4,4-trifluoro-(3R)-**hydroxybutyric** acid ethylester was fermented using E.coli JM109/pKAR,pKKGDH and 4,4,4-trifluoroacetoacetate ethylester as substrate.

IT **53-57-6**, NADPH **367-93-1**, IPTG **9028-12-0**, Aldehyde reductase **37250-50-3**, Dehydrogenase, glucose (nicotinamide adenine dinucleotide phosphate)
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(biotransformation for producing trifluoro-3(R)-**hydroxybutyric** acid derivs. using genetically engineered E.coli)

RN 53-57-6 HCAPLUS

CN Adenosine 5'-(trihydrogen diphosphate), 2'-(dihydrogen phosphate), P'.fwdarw.5'-ester with 1,4-dihydro-1-.beta.-D-ribofuranosyl-3-pyridinecarboxamide (9CI) (CA INDEX NAME)

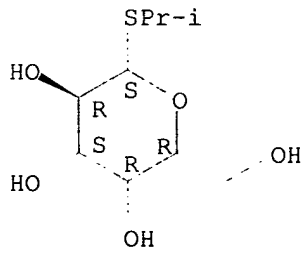
Absolute stereochemistry.



RN 367-93-1 HCAPLUS

CN .beta.-D-Galactopyranoside, 1-methylethyl 1-thio- (9CI) (CA INDEX NAME)

Absolute stereochemistry.

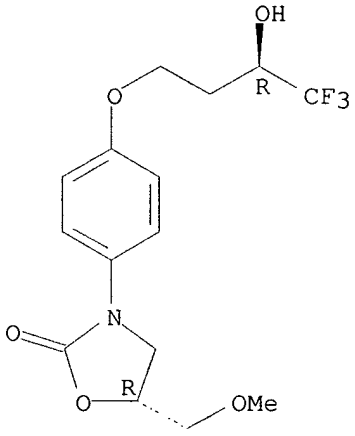


RN 9028-12-0 HCAPLUS
CN Dehydrogenase, alcohol (nicotinamide adenine dinucleotide phosphate) (9CI) (CA INDEX NAME)

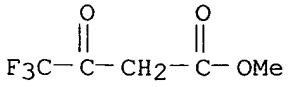
*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
RN 37250-50-3 HCAPLUS
CN Dehydrogenase, glucose (nicotinamide adenine dinucleotide phosphate) (9CI) (CA INDEX NAME)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
IT **134564-82-2P**, Befloxatone
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); SPN (Synthetic preparation); BIOL (Biological study); PREP (Preparation)
(biotransformation for producing trifluoro-3(R)-**hydroxybutyric** acid derivs. using genetically engineered E.coli)
RN 134564-82-2 HCAPLUS
CN 2-Oxazolidinone, 5-(methoxymethyl)-3-[4-[(3R)-4,4,4-trifluoro-3-hydroxybutoxy]phenyl]-, (5R)- (9CI) (CA INDEX NAME)

Absolute stereochemistry.

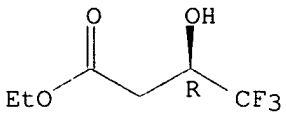


IT **83643-84-9P 85571-85-3P 135548-07-1P**
239133-70-1P 239133-72-3P 239133-73-4P
239133-75-6P 239133-77-8P
RL: BMF (Bioindustrial manufacture); BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(biotransformation for producing trifluoro-3(R)-**hydroxybutyric** acid derivs. using genetically engineered E.coli)
RN 83643-84-9 HCAPLUS
CN Butanoic acid, 4,4,4-trifluoro-3-oxo-, methyl ester (9CI) (CA INDEX NAME)



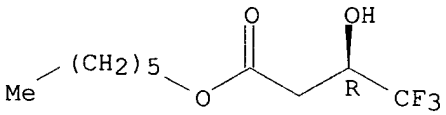
RN 85571-85-3 HCAPLUS
CN Butanoic acid, 4,4,4-trifluoro-3-hydroxy-, ethyl ester, (3R)- (9CI) (CA INDEX NAME)

Absolute stereochemistry. Rotation (+).



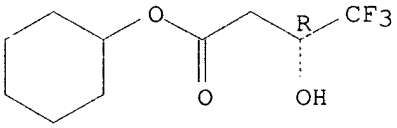
RN 135548-07-1 HCAPLUS
CN Butanoic acid, 4,4,4-trifluoro-3-hydroxy-, hexyl ester, (3R)- (9CI) (CA INDEX NAME)

Absolute stereochemistry.



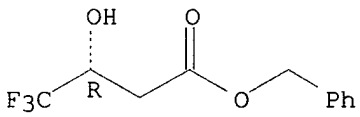
RN 239133-70-1 HCAPLUS
CN Butanoic acid, 4,4,4-trifluoro-3-hydroxy-, cyclohexyl ester, (3R)- (9CI) (CA INDEX NAME)

Absolute stereochemistry.



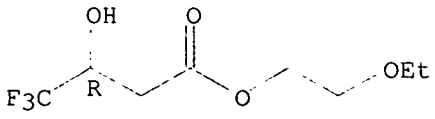
RN 239133-72-3 HCAPLUS
CN Butanoic acid, 4,4,4-trifluoro-3-hydroxy-, phenylmethyl ester, (3R)- (9CI) (CA INDEX NAME)

Absolute stereochemistry.



RN 239133-73-4 HCAPLUS
CN Butanoic acid, 4,4,4-trifluoro-3-hydroxy-, 2-ethoxyethyl ester, (3R)- (9CI) (CA INDEX NAME)

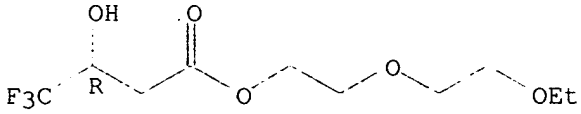
Absolute stereochemistry.



RN 239133-75-6 HCAPLUS
CN Butanoic acid, 4,4,4-trifluoro-3-hydroxy-, 2-(2-ethoxyethoxy)ethyl ester,

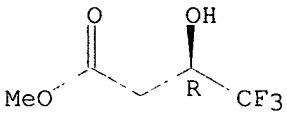
(3R)- (9CI) (CA INDEX NAME)

Absolute stereochemistry.

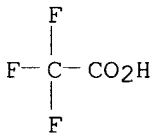


RN 239133-77-8 HCAPLUS
CN Butanoic acid, 4,4,4-trifluoro-3-hydroxy-, methyl ester, (3R)- (9CI) (CA INDEX NAME)

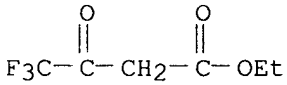
Absolute stereochemistry.



IT **76-05-1D**, Trifluoro acetic acid, derivs., biological studies
372-31-6, Butanoic acid, 4,4,4-trifluoro-3-oxo-, ethyl ester
83097-87-4 239133-69-8 239133-71-2
239133-74-5 239133-76-7
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(biotransformation for producing trifluoro-3(R)-**hydroxybutyric** acid derivs. using genetically engineered E.coli)
RN 76-05-1 HCAPLUS
CN Acetic acid, trifluoro- (8CI, 9CI) (CA INDEX NAME)



RN 372-31-6 HCAPLUS
CN Butanoic acid, 4,4,4-trifluoro-3-oxo-, ethyl ester (9CI) (CA INDEX NAME)



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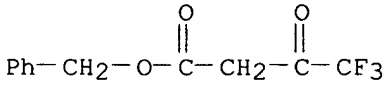

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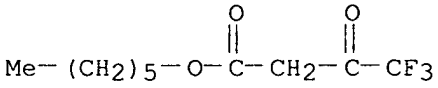

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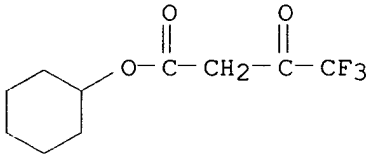

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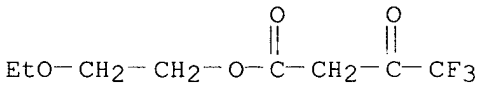

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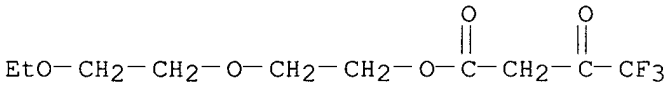


IC ICM C12N015-53
ICS C12P007-42; C12P007-62; C12P011-00; C12P013-02
CC 16-2 (Fermentation and Bioindustrial Chemistry)
ST trifluoro hydroxybutyrate deriv stereoselective redn fermn; fermn Escherichia aldehyde reductase glucose dehydrogenase plasmid befloxatone
IT Chirality
Drugs
Fermentation
Temperature
pH
(biotransformation for producing trifluoro-3(R)-**hydroxybutyric** acid derivs. using genetically engineered E.coli)
IT Intermediates
RL: BMF (Bioindustrial manufacture); BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(biotransformation for producing trifluoro-3(R)-**hydroxybutyric** acid derivs. using genetically engineered E.coli)
IT Plasmid vectors

(pKAR, coding for NADPH dependent aldehyde reductase, from Sporobolomyces salmonicolor; biotransformation for producing trifluoro-3(R)-**hydroxybutyric** acid derivs. using genetically engineered E.coli)

IT Plasmid vectors
(pKKGDH, coding for NADPH-generating glucose dehydrogenase, Ptac and Km resistance; biotransformation for producing trifluoro-3(R)-**hydroxybutyric** acid derivs. using genetically engineered E.coli)

IT Sporobolomyces salmonicolor
(source of pKAR plasmid coding for NADPH dependent aldehyde reductase; biotransformation for producing trifluoro-3(R)-**hydroxybutyric** acid derivs. using genetically engineered E.coli)

IT Bacillus megaterium
(source of pKKGDH plasmid; biotransformation for producing trifluoro-3(R)-**hydroxybutyric** acid derivs. using genetically engineered E.coli)

IT Reduction
(stereoselective; biotransformation for producing trifluoro-3(R)-**hydroxybutyric** acid derivs. using genetically engineered E.coli)

IT Escherichia coli
(strains JM109 or HB 101, host cells, expressing pKAR and pKKGDH; biotransformation for producing trifluoro-3(R)-**hydroxybutyric** acid derivs. using genetically engineered E.coli)

IT **53-57-6**, NADPH **367-93-1**, IPTG **9028-12-0**, Aldehyde reductase **37250-50-3**, Dehydrogenase, glucose (nicotinamide adenine dinucleotide phosphate)
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(biotransformation for producing trifluoro-3(R)-**hydroxybutyric** acid derivs. using genetically engineered E.coli)

IT **134564-82-2P**, Befloxatone
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); SPN (Synthetic preparation); BIOL (Biological study); PREP (Preparation)
(biotransformation for producing trifluoro-3(R)-**hydroxybutyric** acid derivs. using genetically engineered E.coli)

IT **83643-84-9P 85571-85-3P 135548-07-1P 239133-70-1P 239133-72-3P 239133-73-4P 239133-75-6P 239133-77-8P**
RL: BMF (Bioindustrial manufacture); BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(biotransformation for producing trifluoro-3(R)-**hydroxybutyric** acid derivs. using genetically engineered E.coli)

IT **76-05-1D**, Trifluoro acetic acid, derivs., biological studies **76-05-1D**, Trifluoro acetic acid, derivs. **372-31-6**, Butanoic acid, 4,4,4-trifluoro-3-oxo-, ethyl ester **83097-87-4 239133-69-8 239133-71-2 239133-74-5 239133-76-7**
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(biotransformation for producing trifluoro-3(R)-**hydroxybutyric** acid derivs. using genetically engineered E.coli)

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d ibib abs hitstr 2-9

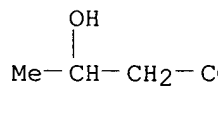
L12 ANSWER 2 OF 9 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1989:552014 HCAPLUS
DOCUMENT NUMBER: 111:152014
TITLE: Mass production of intracellular metabolite by fully automatic fed-batch culture of microorganism
AUTHOR(S): Yamane, Tsuneo; Suzuki, Takahiro; **Shimizu, Shoichi**
CORPORATE SOURCE: Sch. Agric., Nagoya Univ., Nagoya, 464-01, Japan
SOURCE: Bioprod. Bioprocesses, Conf. Promote Jpn./U.S. Jt. Proj. Coop. Biotechnol., 2nd (1989), Meeting Date 1986, 321-36. Editor(s): Fiechter, Armin; Okada, Hirosuke; Tanner, Robert D. Springer: Berlin, Fed. Rep. Ger.
CODEN: 56QOAP
DOCUMENT TYPE: Conference
LANGUAGE: English

AB Attempts were made to produce 2 kinds of intracellular metabolites, poly-.beta.-**hydroxybutyric** acid (PHB) and thiostrepton (TS), by automatic fed-batch cultures at high cell mass concns. At 170 h of cultivation of a methylotroph, 150 g PHB/L (its cellular content was .apprx.64%) was obtained from MeOH with 20% yield. To maintain PHB synthetic activity at a high level, the ratio of MeOH and NH3 (C/N ratio of feed) was gradually raised according to the increase in PHB content with a computer-aided automatic feeding system. At 220 h of cultivation of Streptomyces laurentii, 10.5 g TS/L (its cellular content was .apprx.7%) was obtained from glucose, corn steep liquor, and defatted soybean meal. To keep high TS prodn. rate and to avoid the degrdn. of TS formed, a soln. of these nutrients whose compn. had carefully been detd. exptl. was automatically supplied with a pH-stat mode. A general equation of direct cost for intracellular metabolite prodn. composed of both yield and productivity was proposed. Based on the cost equation, advantages of the fed-batch culture at high cell mass concn. over conventional batch culture are discussed concerning intracellular metabolite prodn.

IT **26063-00-3P**, Poly-.beta.-hydroxybutyrate
RL: BMF (Bioindustrial manufacture); BIOL (Biological study); PREP (Preparation)
(manuf. of, by fed-batch fermn. with Protomonas extorquens at high cell concns.)
RN 26063-00-3 HCAPLUS
CN Butanoic acid, 3-hydroxy-, homopolymer (9CI) (CA INDEX NAME)

CM 1

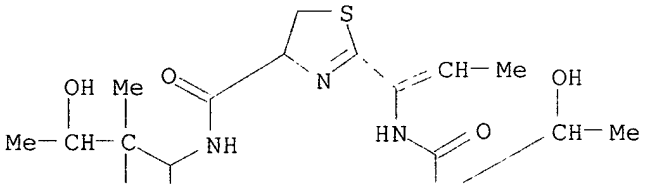
CRN 300-85-6
CMF C4 H8 O3



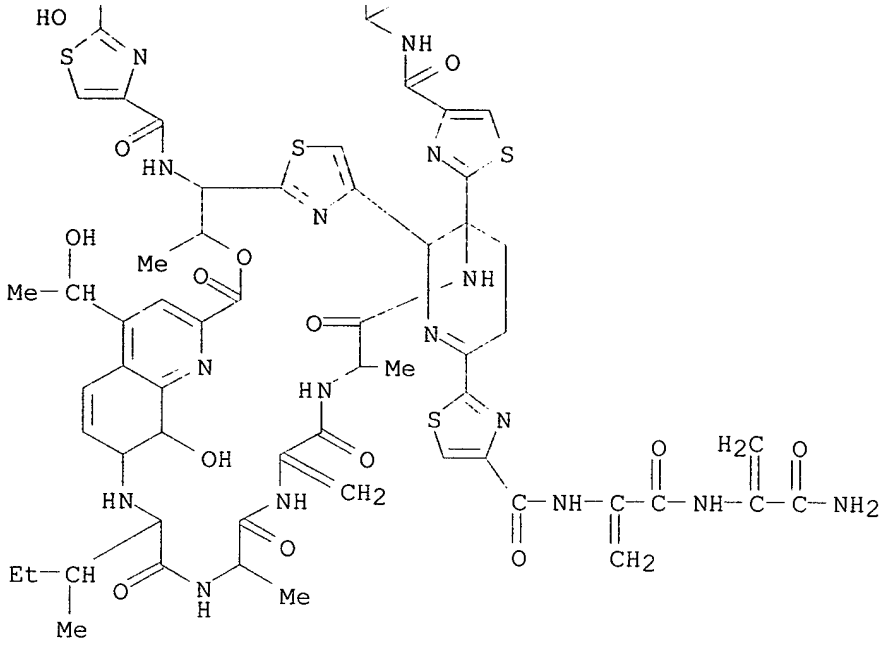
IT **1393-48-2P**, Thiostrepton
RL: BMF (Bioindustrial manufacture); BIOL (Biological study); PREP (Preparation)
(manuf. of, by fed-batch fermn. with Streptomyces laurentii at high cell concns.)
RN 1393-48-2 HCAPLUS
CN Alaninamide, N-[[2-[21-(1,2-dihydroxy-1-methylpropyl)-14-ethylidene-3,9,10,11,12,13,14,18,19,20,21,27,28,32a,39,40-hexadecahydro-39-hydroxy-

11,43-bis(1-hydroxyethyl)-34,49-dimethyl-52-methylene-46-(1-methylpropyl)-9,12,19,26,36,47,50,53,56-nonaoxo-17H,26H-4a,28-(iminoethaniminoethaniminoethaniminoethanimino[7,2]quinolinomethanoxymethano)-8,5:18,15:25,22:32,29-tetranitrilo-4H,15H-pyrido[3,2-m][1,11,17,24,4,7,20,27]tetrathiatetraazacyclotriacontin-2-yl]-4-thiazolyl]carbonyl]-2,3-didehydroalanyl-2,3-didehydro- (9CI) (CA INDEX NAME)

PAGE 1-A



PAGE 2-A



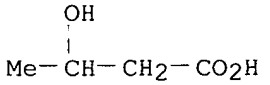
L12 ANSWER 3 OF 9 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1989:93447 HCAPLUS
DOCUMENT NUMBER: 110:93447
TITLE: Production of poly-.beta.-**hydroxybutyric** acid from methanol by microorganisms
AUTHOR(S): **Shimizu, Shoichi**; Suzuki, Takahiro
CORPORATE SOURCE: Fac. Agric., Nagoya Univ., Nagoya, 464, Japan
SOURCE: Hakko to Kogyo (1987), 45(11), 1080-7
CODEN: HAKOD4; ISSN: 0386-0701
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Japanese
AB A review, with 11 refs., on the microbial prodn. of poly-.beta.-**hydroxybutyric** acid from methanol.
IT **67-56-1**, Methanol, biological studies
RL: BIOL (Biological study)
(fermn. of, to **polyhydroxybutyric** acid)
RN 67-56-1 HCAPLUS
CN Methanol (8CI, 9CI) (CA INDEX NAME)

H_3C-OH

IT **26063-00-3P**, Poly-.beta.-**hydroxybutyric** acid
RL: BMF (Bioindustrial manufacture); BIOL (Biological study); PREP (Preparation)
(manuf. of, from methanol by fermn.)
RN 26063-00-3 HCAPLUS
CN Butanoic acid, 3-hydroxy-, homopolymer (9CI) (CA INDEX NAME)

CM 1

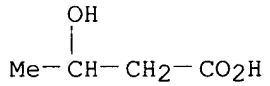
CRN 300-85-6
CMF C4 H8 O3



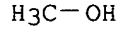
L12 ANSWER 4 OF 9 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1988:148861 HCAPLUS
DOCUMENT NUMBER: 108:148861
TITLE: Control of molecular weight of poly-.beta.-**hydroxybutyric** acid produced in fed-batch culture of Protomonas extorquens
AUTHOR(S): Suzuki, Takahiro; Deguchi, Hiroyuki; Yamane, Tsuneo; **Shimizu, Shoichi**; Gekko, Kunihiko
CORPORATE SOURCE: Fac. Agric., Nagoya Univ., Nagoya, 464, Japan
SOURCE: Applied Microbiology and Biotechnology (1988), 27(5-6), 487-91
CODEN: AMBIDG; ISSN: 0175-7598
DOCUMENT TYPE: Journal
LANGUAGE: English
AB To control mol. wt. of poly-.beta.-**hydroxybutyric** acid (PHB) produced in a fed-batch culture of P. extorquens, the effects of cultural temp., pH, molar ratio of MeOH and NH3, and concn. of MeOH on polymn. were investigated. MeOH concn. affected the av. mol. wt. of PHB. When the cultivation was carried out at 0.05 g MeOH/L, the av. mol. wt. of PHB was >8 .times. 105. On the other hand, with 32 g MeOH/L, the av. mol. wt. of PHB was <0.5 .times. 105. Although every sample had a wide mol. wt. distribution, it became possible to control the av. mol. wt. of PHB.
IT **26063-00-3P**, Poly-.beta.-**hydroxybutyric** acid
RL: BMF (Bioindustrial manufacture); BIOL (Biological study); PREP (Preparation)
(manuf. of, by Protomonas extorquens, control of mol. wt. in)
RN 26063-00-3 HCAPLUS
CN Butanoic acid, 3-hydroxy-, homopolymer (9CI) (CA INDEX NAME)

CM 1

CRN 300-85-6
CMF C4 H8 O3



IT **67-56-1**, Methanol, biological studies
RL: BIOL (Biological study)
(**polyhydroxybutyric** acid mol. wt. control by, during fermn. by Protomonas extorquens)
RN 67-56-1 HCAPLUS
CN Methanol (8CI, 9CI) (CA INDEX NAME)



L12 ANSWER 5 OF 9 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1987:405794 HCAPLUS
DOCUMENT NUMBER: 107:5794
TITLE: Manufacture of poly-.beta.-**hydroxybutyric** acid by Protomonas extorquence
INVENTOR(S): **Shimizu, Shoichi**; Yamane, Tsuneo; Suzuki, Takahiro
PATENT ASSIGNEE(S): Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 26 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 62055094	A2	19870310	JP 1985-193078	19850903
JP 03065154	B4	19911009		

PRIORITY APPLN. INFO.: JP 1985-193078 19850903

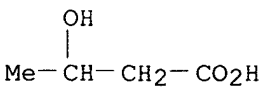
AB Poly-.beta.-**hydroxybutyric** acid (I) is manufd. from cells of Protomonas extorquens K cultivated in concn. using MeOH as a C source. Thus, the microorganism was cultivated in a jar fermentor in medium contg. KH2PO4, Na2HPO4, (NH4)2SO4, MgSO4, FeSO4, CaCl2, MnSO4, CoCl2, ZnSO4, CuCl2, and MeOH at 30.degree. for 160 h, maintaining MeOH 0.5 g/L, pH 7. The culture yielded cells 217 g/L and I 137 g/L.

IT **67-56-1**, Methanol, biological studies
RL: BIOL (Biological study)
(in polyhydroxybutyrate manuf., by Protomonas extorquens)
RN 67-56-1 HCAPLUS
CN Methanol (8CI, 9CI) (CA INDEX NAME)

IT **26063-00-3P**, Poly-.beta.-**hydroxybutyric** acid
RL: BMF (Bioindustrial manufacture); BIOL (Biological study); PREP (Preparation)
(manuf. of, by Protomonas extorquens, methanol in)
RN 26063-00-3 HCAPLUS
CN Butanoic acid, 3-hydroxy-, homopolymer (9CI) (CA INDEX NAME)

CM 1

CRN 300-85-6
CMF C4 H8 O3



L12 ANSWER 6 OF 9 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1986:570589 HCAPLUS
DOCUMENT NUMBER: 105:170589

TITLE: Mass production of poly-.beta.-**hydroxybutyric** acid by fed-batch culture with controlled carbon/nitrogen feeding

AUTHOR(S): Suzuki, Takahiro; Yamane, Tsuneo; **Shimizu, Shoichi**

CORPORATE SOURCE: Fac. Agric., Nagoya Univ., Nagoya, 464, Japan

SOURCE: Applied Microbiology and Biotechnology (1986), 24(5), 370-4
CODEN: AMBIDG; ISSN: 0175-7598

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The effect of the ratio of methanol [**67-56-1**] to ammonia (the C/N ratio in the feeding soln) on microbial poly-.beta.-**hydroxybutyric** acid (PHB) [**26063-00-3**] prodn. was investigated. A const. C/N ratio regulated both the PHB content and the specific rate of PHB prodn. To produce the max. PHB effectively in a short time, the C/N ratio should be controlled automatically according to the increase in PHB content. Variation of the PHB content was estd. by tracing the time-course of CO2 concn. in the exhaust gas. When the cell concn. reached 70 g/L, C/N ratio was gradually increased from the initial C/N ratio of 10 (mol methanol/mol ammonia). At 121 h, concn. of PHB reached 136 g/L, which was the max. level so far obtained. The reaction time was considerably shortened compared with a previous study (175 h). PHB concn. reached 149 g/L at 170 h and total cell concn. became 233 g/L. PHB yield from methanol was 0.20 (g PHB/g methanol), which was also superior to the previous result of 0.18. Fermn. was carried out by Protomonas extorquens.

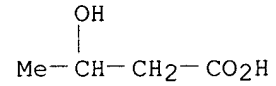
IT **26063-00-3P**
RL: BMF (Bioindustrial manufacture); BIOL (Biological study); PREP (Preparation)
(manuf. of, by Protomonas extorquens in fed-batch culture, carbon/nitrogen feeding effect on)

RN 26063-00-3 HCAPLUS

CN Butanoic acid, 3-hydroxy-, homopolymer (9CI) (CA INDEX NAME)

CM 1

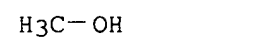
CRN 300-85-6
CMF C4 H8 O3



IT **67-56-1**, biological studies
RL: BIOL (Biological study)
(**polyhydroxybutyric** acid manuf. by Protomonas extorquens response to ammonia and)

RN 67-56-1 HCAPLUS

CN Methanol (8CI, 9CI) (CA INDEX NAME)



IT **7664-41-7**, biological studies
RL: BIOL (Biological study)
(**polyhydroxybutyric** acid manuf. by Protomonas extorquens

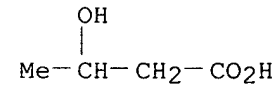
response to methanol and)
RN 7664-41-7 HCAPLUS
CN Ammonia (8CI, 9CI) (CA INDEX NAME)

NH_3

L12 ANSWER 7 OF 9 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1986:570588 HCAPLUS
DOCUMENT NUMBER: 105:170588
TITLE: Kinetics and effect of nitrogen source feeding on production of poly-.beta.-**hydroxybutyric** acid by fed-batch culture
AUTHOR(S): Suzuki, Takahiro; Yamane, Tsuneo; **Shimizu, Shoichi**
CORPORATE SOURCE: Fac. Agric., Nagoya Univ., Nagoya, 464, Japan
SOURCE: Applied Microbiology and Biotechnology (1986), 24(5), 366-9
CODEN: AMBIDG; ISSN: 0175-7598
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A kinetic study of the prodn. of poly-.beta.-**hydroxybutyric** acid (PHB) [**26063-00-3**] by a fed-batch culture of Protomonas extorquens showed that a nitrogen source was necessary even in the PHB prodn. phase. The effect of ammonia feeding on PHB prodn. was consequently investigated. The nitrogen source (ammonia water) was supplied at a low const. feeding rate after the growth phase in which cell mass concn. reached 60 g/L. Feeding with a small quantity of ammonia resulted in a more rapid increase in intracellular PHB content than was the case without ammonia feeding. Excessive feeding of ammonia, however, caused not only degrdn. of accumulated PHB but also redn. of microbial PHB synthetic activity.
IT **26063-00-3P**
RL: BMF (Bioindustrial manufacture); BIOL (Biological study); PREP (Preparation)
(manuf. of, by Protomonas extorquens in feed-batch culture, ammonia feeding effect on)
RN 26063-00-3 HCAPLUS
CN Butanoic acid, 3-hydroxy-, homopolymer (9CI) (CA INDEX NAME)

CM 1

CRN 300-85-6
CMF C4 H8 O3



IT **7664-41-7**, biological studies
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(**polyhydroxybutyric** acid manuf. by Protomonas extorquens response to)
RN 7664-41-7 HCAPLUS
CN Ammonia (8CI, 9CI) (CA INDEX NAME)

NH_3

L12 ANSWER 8 OF 9 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1986:459469 HCAPLUS
DOCUMENT NUMBER: 105:59469
TITLE: Poly(.beta.-**hydroxybutyric** acid) from methanol using Pseudomonas
INVENTOR(S): **Shimizu, Shoichi**; Yamane, Tsuneo; Suzuki, Takahiro
PATENT ASSIGNEE(S): Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 4 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 61070991	A2	19860411	JP 1984-190521	19840913
JP 04063676	B4	19921012		

PRIORITY APPLN. INFO.: JP 1984-190521 19840913

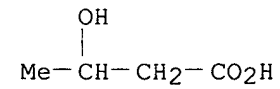
AB Poly(.beta.-**hydroxybutyric** acid) was produced by cultivating P. methanolytica, P. methylovorans, P. methanocola, P. methanoalbum, P. methylica, or P. methanophilum in the presence of 0.1-1.0 g/MeOH/L and 0.05-0.2 g/NH4OH/L at the 1st stage and then cultivating under N-deficient conditions. Thus, a preculture of P. methanophilum was inoculated to a basal medium contg. KH2PO4 0.8, Na2HPO4 3.0, (NH4)2SO4 0.8 g/L, and Mg, Ca, Fe, Zn, Mn, Co, Cu, and Mo. Cultivation at 30.degree. for 144 h while feeding MeOH (.apprx.0.5 g/L concn. kept), NH4OH (stopped after 75 h), H3PO4, and minerals gave 207 g cells/L contg. the title polymer in 64% yield.

IT **26063-00-3P**
RL: PREP (Preparation)
(manuf. of with Pseudomonas)

RN 26063-00-3 HCAPLUS
CN Butanoic acid, 3-hydroxy-, homopolymer (9CI) (CA INDEX NAME)

CM 1

CRN 300-85-6
CMF C4 H8 O3



L12 ANSWER 9 OF 9 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1986:166869 HCAPLUS
DOCUMENT NUMBER: 104:166869
TITLE: Mass production of poly-.beta.-**hydroxybutyric** acid by fully automatic fed-batch culture of methylotroph

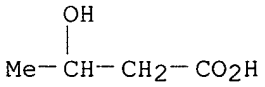
AUTHOR(S): Suzuki, Takahiro; Yamane, Tsuneo; **Shimizu, Shoichi**
CORPORATE SOURCE: Sch. Agric., Nagoya Univ., Nagoya, 464, Japan
SOURCE: Applied Microbiology and Biotechnology (1986), 23(5), 322-9
CODEN: AMBIDG; ISSN: 0175-7598
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A Pseudomonas was selected from 51 methylotrophs for its prodn. of poly-.beta.-**hydroxybutyric** acid (PHB) [**26063-00-3**] from MeOH [**67-56-1**]. Fermn. was by microcomputer-aided fully automatic fed-batch culture. Temp., dissolved O2 concn. (DO), and MeOH concn. were maintained at 30.degree., 2.5 ppm, and 0.5 g/L, resp. N source and minerals were also controlled to maintain their initial concns. during cell growth. When a high cell concn. was achieved (160 g/L), NH3 and minerals were stopped, and only MeOH was supplied. At 175 h, a high concn. of PHB (136 g/L) was obtained, and total cell concn. became 206 g/L. DO must be maintained above the crit. level during the PHB formation phase. PHB yield was 0.18 g/g MeOH, and the max. PHB content reached 66% of dry wt. Solid PHB had a m.p. of 176.degree. and an av. mol. wt. of 3.0 .times. 105.

IT **67-56-1**, biological studies
RL: BIOL (Biological study)
(fermn. of, to polyhydroxybutyrate with Pseudomonas)
RN 67-56-1 HCAPLUS
CN Methanol (8CI, 9CI) (CA INDEX NAME)

IT **26063-00-3P**
RL: BMF (Bioindustrial manufacture); BIOL (Biological study); PREP (Preparation)
(manuf. of, from methanol with Pseudomonas)
RN 26063-00-3 HCAPLUS
CN Butanoic acid, 3-hydroxy-, homopolymer (9CI) (CA INDEX NAME)

CM 1

CRN 300-85-6
CMF C4 H8 O3



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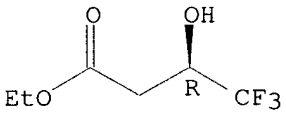
L33 ANSWER 1 OF 2 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:102198 HCAPLUS
DOCUMENT NUMBER: 134:326169
TITLE: Novel unusual microbial dehalogenation during enantioselective reduction of ethyl 4,4,4-trifluoroacetoacetate with baker's yeast
AUTHOR(S): Bertau, M.
CORPORATE SOURCE: Institut fur Biochemie, Technische Universitat Dresden, Dresden, 01062, Germany
SOURCE: Tetrahedron Letters (2001), 42(7), 1267-1268
CODEN: TELEAY; ISSN: 0040-4039
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
OTHER SOURCE(S): CASREACT 134:326169

AB In the course of investigating microbial syntheses for chiral pharmaceutical intermediates, CF3COCH2CO2Et was submitted to baker's yeast redn. To obtain the D-carbinol in high enantiopurity, several additives were tested for L-reductase inhibitor activity. Allyl alc. proved to be not only a suitable additive, but also an inducer for effective defluorination of the substrate.

IT **85571-85-3P 99437-70-4P**
RL: BPN (Biosynthetic preparation); BIOL (Biological study); **PREP (Preparation)**
(microbial defluorination during asym. redn. of trifluoroacetoacetate with baker's yeast)

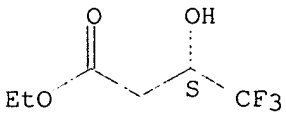
RN 85571-85-3 HCAPLUS
CN Butanoic acid, 4,4,4-trifluoro-3-hydroxy-, ethyl ester, (3R)- (9CI) (CA INDEX NAME)

Absolute stereochemistry. Rotation (+).



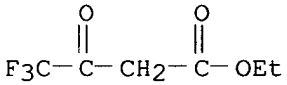
RN 99437-70-4 HCAPLUS
CN Butanoic acid, 4,4,4-trifluoro-3-hydroxy-, ethyl ester, (3S)- (9CI) (CA INDEX NAME)

Absolute stereochemistry. Rotation (-).



IT **372-31-6**, Ethyl 4,4,4-trifluoroacetoacetate
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(microbial defluorination during asym. redn. of trifluoroacetoacetate with baker's yeast)

RN 372-31-6 HCAPLUS
CN Butanoic acid, 4,4,4-trifluoro-3-oxo-, ethyl ester (9CI) (CA INDEX NAME)



REFERENCE COUNT: 12 THERE ARE 12 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d ibib abs hitstr 2

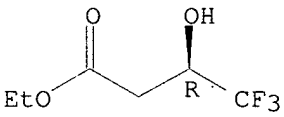
L33 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1999:461885 HCAPLUS
DOCUMENT NUMBER: 131:242011
TITLE: (R)-(+) and (S)-(-) ethyl 4,4,4-trifluoro-3-hydroxy butanoate by enantioselective Baker's yeast reduction
AUTHOR(S): Davoli, Paolo; Forni, Arrigo; Moretti, Irene; Prati, Fabio; Torre, Giovanni
CORPORATE SOURCE: Dipartimento di Chimica, Universita di Modena, Modena, 41100, Italy
SOURCE: Enzyme and Microbial Technology (1999), 25(1/2), 149-152
CODEN: EMTED2; ISSN: 0141-0229
PUBLISHER: Elsevier Science Ireland Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB (R)-(+) and (S)-(-) Et 4,4,4-trifluoro-3-hydroxybutanoate are obtained both by enantioselective Baker's yeast redn. of Et 4,4,4-trifluoro-3-oxobutanoate in the presence of allyl bromide or allyl alc. The two additives act as inhibitors of Si or Re yeast-**enzymes**, resp.

IT **85571-85-3P 99437-70-4P**
RL: BPN (Biosynthetic preparation); BIOL (Biological study); **PREP (Preparation)**
(synthesis of Et trifluorohydroxybutanoate enantiomers by stereochem. redn. using baker's yeast)

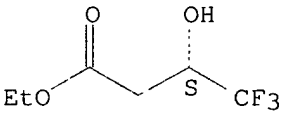
RN 85571-85-3 HCAPLUS
CN Butanoic acid, 4,4,4-trifluoro-3-hydroxy-, ethyl ester, (3R)- (9CI) (CA INDEX NAME)

Absolute stereochemistry. Rotation (+).



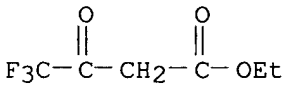
RN 99437-70-4 HCAPLUS
CN Butanoic acid, 4,4,4-trifluoro-3-hydroxy-, ethyl ester, (3S)- (9CI) (CA INDEX NAME)

Absolute stereochemistry. Rotation (-).



IT **372-31-6**, Ethyl 4,4,4-trifluoro-3-oxobutanoate
RL: BPR (Biological process); BSU (Biological study, unclassified); **RCT (Reactant)**; BIOL (Biological study); PROC (Process); RACT (Reactant or reagent)
(synthesis of Et trifluorohydroxybutanoate enantiomers by stereochem. redn. using baker's yeast)

RN 372-31-6 HCAPLUS
CN Butanoic acid, 4,4,4-trifluoro-3-oxo-, ethyl ester (9CI) (CA INDEX NAME)



REFERENCE COUNT: 19 THERE ARE 19 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

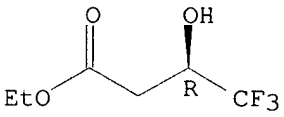
=> d ibib abs hitstr 1-2

L35 ANSWER 1 OF 2 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:326792 HCAPLUS
DOCUMENT NUMBER: 137:46794
TITLE: Efficient enantioselective reduction of ketones with Daucus carota root
AUTHOR(S): Yadav, J. S.; Nanda, S.; Reddy, P. Thirupathi; Rao, A. Bhaskar
CORPORATE SOURCE: Organic Division, Indian Institute of Chemical Technology, Hyderabad, 500007, India
SOURCE: Journal of Organic Chemistry (2002), 67(11), 3900-3903
CODEN: JOCEAH; ISSN: 0022-3263
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
OTHER SOURCE(S): CASREACT 137:46794

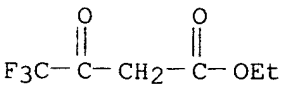
AB A novel and efficient redn. of various prochiral ketones such as acetopehones, .alpha.-azido aryl ketones, .beta.-keto esters, and aliph. acyclic and cyclic ketones to the corresponding optically active secondary alcs. with moderate to excellent chem. yield was achieved by using Daucus carota, root plant **cells** under extremely mild and environmentally benign conditions in aq. medium, has been described. Many of these optically active alcs. are the potential chiral building blocks for the synthesis of pharmaceutically important mols. and asym. chiral ligands. Hence, this biocatalytic approach is found to be the most suitable for the prepn. of a wide range of chiral alcs. and gave inspiration for the development of a new biotechnol. process.

IT **85571-85-3P**
RL: BPN (Biosynthetic preparation); BIOL (Biological study); **PREP (Preparation)**
(enantioselective redn. of ketones with Daucus carota root)
RN 85571-85-3 HCAPLUS
CN Butanoic acid, 4,4,4-trifluoro-3-hydroxy-, ethyl ester, (3R)- (9CI) (CA INDEX NAME)

Absolute stereochemistry. Rotation (+).



IT **372-31-6**
RL: **RCT (Reactant)**; RACT (Reactant or reagent)
(enantioselective redn. of ketones with Daucus carota root)
RN 372-31-6 HCAPLUS
CN Butanoic acid, 4,4,4-trifluoro-3-oxo-, ethyl ester (9CI) (CA INDEX NAME)



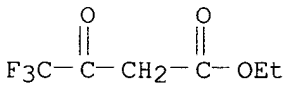
REFERENCE COUNT: 56 THERE ARE 56 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L35 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1992:192572 HCAPLUS
DOCUMENT NUMBER: 116:192572
TITLE: Preparation of both enantiomers of ethyl 4,4,4-trifluoro-3-hydroxy butanoate by enantioselective **microbial** reduction
AUTHOR(S): Guerrero, A.; Raja, F.
CORPORATE SOURCE: Dep. Biol. Org. Chem., CID, Barcelona, 08034, Spain
SOURCE: Bioorganic & Medicinal Chemistry Letters (1991), 1(12), 675-8
CODEN: BMCLE8; ISSN: 0960-894X
DOCUMENT TYPE: Journal
LANGUAGE: English

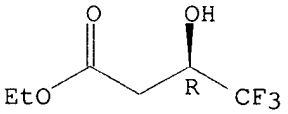
AB The effect of some parameters, i.e. temp., time, pH and concn., on the baker's yeast redn. of Et 4,4,4-trifluoroacetoacetate is presented. The enantiomeric excess of the R enantiomer appeared to increase up to 76% when the temp. of the redn. decreased. The other factors do not appear to improve the enantioselectivity of the reaction. Redn. with Candida utilis allowed prepn. of the S enantiomer in higher optical purity than previously reported.

IT **372-31-6**, Ethyl 4,4,4-trifluoroacetoacetate
RL: **RCT (Reactant)**; RACT (Reactant or reagent)
(enantioselective redn. of, to trifluorohydroxybutanoate by yeast)
RN 372-31-6 HCAPLUS
CN Butanoic acid, 4,4,4-trifluoro-3-oxo-, ethyl ester (9CI) (CA INDEX NAME)



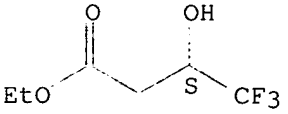
IT **85571-85-3P 99437-70-4P**
RL: **PREP (Preparation)**
(prepn. of, by enantioselective redn. of Et trifluoroacetoacetate by yeast)
RN 85571-85-3 HCAPLUS
CN Butanoic acid, 4,4,4-trifluoro-3-hydroxy-, ethyl ester, (3R)- (9CI) (CA INDEX NAME)

Absolute stereochemistry. Rotation (+).



RN 99437-70-4 HCAPLUS
CN Butanoic acid, 4,4,4-trifluoro-3-hydroxy-, ethyl ester, (3S)- (9CI) (CA INDEX NAME)

Absolute stereochemistry. Rotation (-).



Inv. search w/ E. coli

=> d ibib abs hitstr 1-18

L41 ANSWER 1 OF 18 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2003:6150 HCAPLUS
DOCUMENT NUMBER: 138:38160
TITLE: Production of optically active (R)-2-chloro-1-(3'-chlorophenyl)ethanol by enzymic resolution
INVENTOR(S): **Shimizu, Sakayu**; Kataoka, Michihiko; Kizaki, Noriyuki; Yasohara, Yoshihiko
PATENT ASSIGNEE(S): Kaneka Corporation, Japan
SOURCE: PCT Int. Appl., 23 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003000911	A1	20030103	WO 2002-JP6343	20020625
W: CZ, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR				
JP 2003000290	A2	20030107	JP 2001-191517	20010625

PRIORITY APPLN. INFO.: JP 2001-191517 A 20010625
AB The optically active (R)-2-chloro-1-(3'-chlorophenyl)ethanol, which is useful as a material for the synthesis of medicines, agricultural chems., is com. manufd. from 2-chloro-1-(3'-chlorophenyl)ethanone by stereoselective **redn**. using microorganism such as Escherichia.
REFERENCE COUNT: 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L41 ANSWER 2 OF 18 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:696139 HCAPLUS
DOCUMENT NUMBER: 137:228597
TITLE: Aminoketone asymmetric **reductase** from Rhodococcus erythropolis synthesizing d-pseudoephedrine from 1-2-methylaminopropiophenone, gene, and use in stereoselective synthesis of amino alcohols
INVENTOR(S): Sakamoto, Keiji; Kita, Shinji; Tsuzaki, Kazuya; Morikawa, Tadanori; **Shimizu, Sakayu**; Kataoka, Michihiko
PATENT ASSIGNEE(S): Daiichi Fine Chemical Co., Ltd., Japan
SOURCE: PCT Int. Appl., 79 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002070714	A1	20020912	WO 2002-JP1928	20020301
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU,				

TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
PRIORITY APPLN. INFO.: JP 2001-58698 A 20010302
OTHER SOURCE(S): MARPAT 137:228597
AB An aminoketone asym. **reductase** acting on 1-2-methylaminopropiophenone to form d-pseudoephedrine, from Rhodococcus erythropolis, gene, recombinant expression, and use in enzymic synthesis of optically active amino alcs., are disclosed. The aminoketone asym. **reductase** have the following physicochem. properties: substrate: 1-2-methylaminopropiophenone; optimum pH value: 8.1; optimum temp.: 55.degree.; coenzyme: NADP; and mol. wt.: homotetramer of about 28500 Da. It also acts on 1-2-amino-2-hydroxypropane, 1-2-dimethylaminopropiophenone, 1-amino-2-butanone. The enzyme activity is inhibited by .alpha.,.alpha.'-dipyridyl, o-phenanthroline, and EDTA. A gene coding for it was cloned from Rhodococcus erythropolis strain MAK-34 and its sequence detd.
REFERENCE COUNT: 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L41 ANSWER 3 OF 18 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:731007 HCAPLUS
DOCUMENT NUMBER: 135:271994
TITLE: Pseudomonas ipu operon and recombinant microorganisms for production of L-alaninol and .gamma.-glutamyl amides
INVENTOR(S): Leisinger, Thomas; van der Ploeg, Jan; **Kiener, Andreas M.**; Waesch, Susana Ivone de Azevedo; Maire, Tere
PATENT ASSIGNEE(S): Lonza A.-G., Switz.
SOURCE: PCT Int. Appl., 106 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001073038	A2	20011004	WO 2001-EP3651	20010330
WO 2001073038	A3	20021024		

W: AE, AG, AL, AM, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
PRIORITY APPLN. INFO.: EP 2000-106888 A 20000331
AB Disclosed are novel micro-organisms which are capable of transforming isopropylamine into L-alaninol and wherein the genes ipuH and ipuI coding for enzymes involved in the metabolization of L-alaninol are deactivated. The invention also relates to a method for the prodn. of L-alaninol or theanine using said novel micro-organisms. Thus, the ipuABCDEFGH operon of Pseudomonas was cloned and sequenced. A Pseudomonas ipuH- mutant was used to convert isopropylamine to L-alaninol. E. coli expressing the ipuABCDEFG genes also converted isopropylamine to L-alaninol. The ipuC

gene was cloned and expressed in E. coli. The product, .gamma.-glutamylamide synthetase, was purified and shown to catalyze the formation of theanine from L-glutamic acid and ethylamine. A large no. of other amines were found to be suitable substrates.

L41 ANSWER 4 OF 18 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:454835 HCAPLUS
DOCUMENT NUMBER: 135:179761
TITLE: Synthesis of optically pure ethyl (S)-4-chloro-3-hydroxybutanoate by Escherichia coli transformant cells coexpressing the carbonyl **reductase** and glucose dehydrogenase genes
AUTHOR(S): Kizaki, N.; Yasohara, Y.; Hasegawa, J.; Wada, M.; Kataoka, M.; **Shimizu, S.**
CORPORATE SOURCE: Fine Chemicals Research Laboratories, Kaneka Corporation, Takasago, 676-8688, Japan
SOURCE: Applied Microbiology and Biotechnology (2001), 55(5), 590-595
CODEN: AMBIDG; ISSN: 0175-7598
PUBLISHER: Springer-Verlag
DOCUMENT TYPE: Journal
LANGUAGE: English
OTHER SOURCE(S): CASREACT 135:179761
AB The asym. **redn.** of Et 4-chloro-3-oxobutanoate (COBE) to Et (S)-4-chloro-3-hydroxybutanoate ((S)-CHBE) was investigated. Escherichia coli cells expressing both the carbonyl **reductase** (S1) gene from Candida magnoliae and the glucose dehydrogenase (GDH) gene from Bacillus megaterium were used as the catalyst. In an org.-solvent-water two-phase system, (S)-CHBE formed in the org. phase amounted to 2.58 M (430 g/l), the molar yield being 85%. E. coli transformant cells coproducing S1 and GDH accumulated 1.25 M (208 g/l) (S)-CHBE in an aq. monophase system by continuously feeding on COBE, which is unstable in an aq. soln. In this case, the calcd. turnover of NADP+ (the oxidized form of NADP+) to CHBE was 21,600 mol/mol. The optical purity of the (S)-CHBE formed was 100% enantiomeric excess in both systems. The aq. system used for the **redn.** reaction involving E. coli HB101 cells carrying a plasmid contg. the S1 and GDH genes as a catalyst is simple. Furthermore, the system does not require the addn. of com. available GDH or an org. solvent. Therefore this system is highly advantageous for the practical synthesis of optically pure (S)-CHBE.
REFERENCE COUNT: 19 THERE ARE 19 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L41 ANSWER 5 OF 18 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:753065 HCAPLUS
DOCUMENT NUMBER: 134:53045
TITLE: MioC is an FMN-binding protein that is essential for Escherichia coli biotin synthase activity in vitro
AUTHOR(S): **Birch, Olwen M.**; Hewitson, Kirsty S.; Fuhrmann, Martin; Burgdorf, Knut; Baldwin, Jack E.; Roach, Peter L.; Shaw, Nicholas M.
CORPORATE SOURCE: Biotechnology Research, Lonza A.G., Visp, CH-3930, Switz.
SOURCE: Journal of Biological Chemistry (2000), 275(41), 32277-32280
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Biotin synthase is required for the conversion of dethiobiotin to biotin and requires a no. of accessory proteins and small mol. cofactors for activity in vitro. We have previously identified two of these proteins as flavodoxin and ferredoxin (flavodoxin) NADP+ **reductase**. We now report the identification of MioC as a third essential protein, together with its cloning, purifn., and characterization. Purified MioC has a UV-visible spectrum characteristic of a flavoprotein and contains FMN. The presence of FMN and the primary sequence similarity to flavodoxin suggest that MioC may function as an electron transport protein. The role of MioC in the biotin synthase reaction is discussed, and the structure and function of MioC is compared with that of flavodoxin.
REFERENCE COUNT: 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L41 ANSWER 6 OF 18 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:309430 HCAPLUS
DOCUMENT NUMBER: 132:333410
TITLE: Enzymatic production of chiral compounds using Escherichia coli transformants
AUTHOR(S): Kataoka, Michihiko; Kita, Keiko; **Shimizu, Sakayu**
CORPORATE SOURCE: Grad. Sch. Agric., Kyoto Univ., Japan
SOURCE: Kagaku to Seibutsu (2000), 38(5), 313-318
CODEN: KASEAA; ISSN: 0453-073X
PUBLISHER: Gakkai Shuppan Senta
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Japanese
AB A review with 18 refs. on prodn. of (R)- or (S)-Et 4-chloro-3-hydroxylbutanoate (CHBE) by asym. **redn.** of Et 4-chloro-3-oxobutanoate (COBE) in the presence of Escherichia coli transformants which produce **reductases**, i.e. aldehyde **reductase** (ARI) from Sporobolomyces salmonicolor and carbonyl **reductase** (S1) from Candida magnoliae.

L41 ANSWER 7 OF 18 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:17823 HCAPLUS
DOCUMENT NUMBER: 132:177764
TITLE: Diversity of 4-chloroacetoacetate ethyl ester-**reducing** enzymes in yeasts and their application to chiral alcohol synthesis
AUTHOR(S): Kita, Keiko; Kataoka, Michihiko; **Shimizu, Sakayu**
CORPORATE SOURCE: Department of Biotechnology, Tottori University, Tottori, 680-8552, Japan
SOURCE: Journal of Bioscience and Bioengineering (1999), 88(6), 591-598
CODEN: JBBIF6; ISSN: 1389-1723
PUBLISHER: Society for Bioscience and Bioengineering, Japan
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB Review with 42 refs. Enzymes which **reduce** 4-chloroacetoacetate Et ester (CAAE) to (R)- or (S)-Et 4-chloro-3-hydroxybutanoate (CHBE) were investigated. Several microorganisms which can **reduce** CAAE with high yields were discovered. An NADPH-dependent aldehyde **reductase**, ARI, and an NADPH-dependent carbonyl **reductase**, S1, were isolated from Sporobolomyces salmonicolor and Candida magnoliae, resp., and enzymic synthesis of chiral CHBE was performed through the **redn.** of CAAE. When ARI-overproducing Escherichia coli transformant cells or C. magnoliae cells were incubated in an org. solvent-water diphasic system, CAAE was stoichiometrically converted to

(R)- or (S)-CHBE (>92% enantiomeric excess), resp. Multiple CAAE-**reducing** enzymes were present in S. salmonicolor, C. magnoliae and bakers' yeast. Comparison of the primary structures of these CAAE-**reducing** enzymes with other protein sequences showed that CAAE-**reducing** enzymes are widely distributed in various protein families, and various physiol. roles of these enzymes in the cell were speculated.

REFERENCE COUNT: 42 THERE ARE 42 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L41 ANSWER 8 OF 18 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1999:332377 HCAPLUS
DOCUMENT NUMBER: 131:129076
TITLE: Stereoselective **reduction** of ethyl 4-chloro-3-oxobutanoate by Escherichia coli transformant cells coexpressing the aldehyde **reductase** and glucose dehydrogenase genes
AUTHOR(S): Kataoka, M.; Yamamoto, K.; Kawabata, H.; Wada, M.; Kita, K.; Yanase, H.; **Shimizu, S.**
CORPORATE SOURCE: Graduate Sch. Agric., Kyoto Univ., Kyoto, 606-8502, Japan
SOURCE: Applied Microbiology and Biotechnology (1999), 51(4), 486-490
CODEN: AMBIDG; ISSN: 0175-7598
PUBLISHER: Springer-Verlag
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The asym. **redn.** of Et 4-chloro-3-oxobutanoate to Et (R)-4-chloro-3-hydroxybutanoate (I) using E. coli cells, which coexpress both the aldehyde **reductase** gene from Sporobolomyces salmonicolor and the glucose dehydrogenase (GDH) gene from Bacillus megaterium as a catalyst was investigated. In an org. solvent-water 2-phase system, I formed in the org. phase amounted to 1610 mM (268 mg/mL), with a molar yield of 94.1% and an optical purity of 91.7% e.e. The calcd. turnover no. of NADP+ to I formed was 13,500 mol/mol. Since the use of E. coli JM109 cells harboring pKAR and pACGD as a catalyst is simple and does not require the addn. of GDH or the isolation of the enzymes, it is highly advantageous for the practical synthesis of I.

REFERENCE COUNT: 20 THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L41 ANSWER 9 OF 18 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1998:550496 HCAPLUS
DOCUMENT NUMBER: 129:186143
TITLE: Cloning of gene for a novel carbonyl **reductase** of Candida and characterization and use of the enzyme for producing optically active alcohols
INVENTOR(S): Yasohara, Yoshihiko; Kizaki, Noriyuki; Hasegawa, Junzo; Wada, Masaru; **Shimizu, Sakayu**; Kataoka, Michihiko; Yamamoto, Kazuhiko; Kawabata, Hiroshi; Kita, Keiko
PATENT ASSIGNEE(S): Kaneka Corporation, Japan
SOURCE: PCT Int. Appl., 60 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

WO 9835025	A1	19980813	WO 1997-JP3051	19970901
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9740329	A1	19980826	AU 1997-40329	19970901
EP 967271	A1	19991229	EP 1997-937861	19970901
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
US 6218156	B1	20010417	US 1999-367012	19991124
US 2002006651	A1	20020117	US 2001-777157	20010205
US 6448052	B2	20020910		

PRIORITY APPLN. INFO.:

JP 1997-25667	A	19970207
JP 1997-113052	A	19970430
WO 1997-JP3051	W	19970901
US 1999-367012	A3	19991124

OTHER SOURCE(S): MARPAT 129:186143

AB The gene encoding a novel n carbonyl **reductase** capable of asym. **reducing** a carbonyl compd. R1CH2C(:O)HC(R2)CO2R3 (I; R1=halo; R2=H; R3=(non)substituted alkyl or aryl) to an optically active alc. R1CH2CHOHC(R2)CO2R3 (R1, R2, R3 as in I) is isolated from Candida magnoliae strain IFO0705 and its amino acid sequence deduced. The purifd. enzyme exhibits a pH optimum 5.5-6.5, temp. optimum 50-55, mol. wt. 32,000 by SDS-PAGE or 76,000 by gel filtration. The enzyme specifically **reduces** 4-chloro ethylacetoacetate to (S)-4-Cl-3-hydroxyethylbutyrate in the presence of NADPH and glucose dehydrogenase. Prepn. of transgenic Escherichia coli for the expression of carbonyl **reductase** and glucose dehydrogenase and use of the E. coli for the prodn. of (S)-4-halo-3-hydroxyethylbutyrate was shown.

REFERENCE COUNT: 12 THERE ARE 12 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L41 ANSWER 10 OF 18 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1998:122667 HCAPLUS

DOCUMENT NUMBER: 128:204100

TITLE: Enzymic production of ethyl (R)-4-chloro-3-hydroxybutanoate: asymmetric **reduction** of ethyl 4-chloro-3-oxobutanoate by an Escherichia coli transformant expressing the aldehyde **reductase** gene from yeast

AUTHOR(S): Kataoka, M.; Rohani, L. P. S.; Yamamoto, K.; Wada, M.; Kawabata, H.; Kita, K.; Yanase, H.; **Shimizu, S.**

CORPORATE SOURCE: Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto, 606-01, Japan

SOURCE: Applied Microbiology and Biotechnology (1997), 48(6), 699-703
CODEN: AMBIDG; ISSN: 0175-7598

PUBLISHER: Springer-Verlag

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The asym. **redn.** of Et 4-chloro-3-oxobutanoate (COBE) to Et (R)-4-chloro-3-hydroxybutanoate (CHBE) using Escherichia coli JM109 (pKAR) cells expressing the aldehyde **reductase** gene from Sporobolomyces salmonicolor AKU4429 as a catalyst was studied. The **redn.**

required NADP+, glucose and glucose dehydrogenase for NADPH regeneration. In an aq. system, the substrate was unstable, and inhibition of the reaction by the substrate was also obsd. Efficient conversion of COBE to (R)-CHBE with a satisfactory enantiomeric excess (ee) was attained on incubation with transformant cells in an Bu acetate/water two-phase system contg. the above NADPH-regeneration system. Under the optimized conditions, with the periodical addn. of COBE, glucose and glucose dehydrogenase, the (R)-CHBE yield reached 1530 mM (255 mg/mL) in the org. phase, with a molar conversion yield of 91.1% and an optical purity of 91% ee. The calcd. turnover of NADP+, based on the amts. of NADP+ added and CHBE formed, was about 5100 mol/mol.

L41 ANSWER 11 OF 18 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1998:94500 HCAPLUS
DOCUMENT NUMBER: 128:191632
TITLE: Escherichia coli transformant expressing the glucose dehydrogenase gene from Bacillus megaterium as a cofactor regenerator in a chiral alcohol production system
AUTHOR(S): Kataoka, Michihiko; Rohani, Luh Poni Sri; Wada, Masaru; Kita, Keiko; Yanase, Hideshi; Urabe, Itaru; **Shimizu, Sakayu**
CORPORATE SOURCE: Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto, 606-01, Japan
SOURCE: Bioscience, Biotechnology, and Biochemistry (1998), 62(1), 167-169
CODEN: BBBIEJ; ISSN: 0916-8451
PUBLISHER: Japan Society for Bioscience, Biotechnology, and Agrochemistry
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Escherichia coli JM109 (pGDA2) overexpressing the glucose dehydrogenase (GDH) gene from Bacillus megaterium IWG3 was examd. for use as a cofactor regenerator. In the asym. **redn**. of Et 4-chloro-3-oxobutanoate by E. coli JM109 (pKAR) which is an aldehyde **reductase** -overproducing transformant, E. coli JM109 (pGDA2) can act as an NADPH regenerator with NADP+ and glucose, similarly to com. available GDH.
REFERENCE COUNT: 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L41 ANSWER 12 OF 18 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1996:395452 HCAPLUS
DOCUMENT NUMBER: 125:134092
TITLE: Cloning of the aldehyde **reductase** gene from a red yeast, Sporobolomyces salmonicolor, and characterization of the gene and its product
AUTHOR(S): Kita, Keiko; Matsuzaki, Koji; Hashimoto, Tetsu; Yanase, Hideshi; Kato, Nobuo; Chung, Max Ching-Ming; Kataoka, Michihiko; **Shimizu, Sakayu**
CORPORATE SOURCE: Department Biotechnology, Tottori University, Tottori, 680, Japan
SOURCE: Applied and Environmental Microbiology (1996), 62(7), 2303-2310
CODEN: AEMIDF; ISSN: 0099-2240
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB An NADPH-dependent aldehyde **reductase** (ALR) isolated from a red yeast, Sporobolomyces salmonicolor, catalyzes the **redn**. of a variety of carbonyl compds. To investigate its primary structure, we

cloned and sequenced the cDNA coding for ALR. The aldehyde **reductase** gene (ALR) comprises 969 bp and encodes a polypeptide of 35,232 Da. The deduced amino acid sequence showed a high degree of similarity to other members of the aldo-keto **reductase** superfamily. Anal. of the genomic DNA sequence indicated that the ALR gene was interrupted by six introns (two in the 5' noncoding region and four in the coding region). Southern hybridization anal. of the genomic DNA from S. salmonicolor indicated that there was one copy of the gene. The ALR gene was expressed in Escherichia coli under the control of the tac promoter. The enzyme expressed in E. coli was purified to homogeneity and showed the same catalytic properties as did the enzyme from S. salmonicolor.

L41 ANSWER 13 OF 18 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1995:747118 HCAPLUS
DOCUMENT NUMBER: 123:137068
TITLE: Biotin synthase from Escherichia coli, an investigation of the low molecular weight and protein components required for activity in vitro
AUTHOR(S): **Birch, Olwen M.**; Furhmann, Martin; Shaw, Nicholas M.
CORPORATE SOURCE: Biotechnol. Dep., Lonza A.G., Visp, CH-3930, Switz.
SOURCE: Journal of Biological Chemistry (1995), 270(32), 19158-65
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Bio logy
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The authors have developed a radiochem. method for the measurement of biotin synthase (I) activity in vitro. A cell-free ext. from an E. coli strain contg. a cloned bioB I gene was incubated with [14C]dethiobiotin, which was converted to [14C]biotin. The assay was used to identify the low-mol.-wt. compds. and 2 of the proteins that, in addn. to the bioB gene product, are required for I activity in vitro. The low-mol.-wt. compds. were cysteine; S-adenosylmethionine; thiamin pyrophosphate; Fe2+; a pyridine nucleotide (the most effective being NADPH); and one of the amino acids, asparagine, aspartate, glutamine, or serine. The proteins were flavodoxin and ferredoxin/flavodoxin-NADP **reductase** (EC 1.18.1.2). A 3rd thiamin pyrophosphate-dependent protein was also required for activity. When the cell-free ext. was incubated with nonlabeled dethiobiotin and either [35S]cysteine or [35S]cystine, 35S was incorporated into biotin, and further evidence is presented that cysteine, and not S-adenosylmethionine or methionine, is the S donor for the I reaction.

L41 ANSWER 14 OF 18 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1993:95359 HCAPLUS
DOCUMENT NUMBER: 118:95359
TITLE: Cloning of a .beta.-glucosidase gene from Ruminococcus albus and its expression in Escherichia coli
AUTHOR(S): Ohmiya, Kunio; Takano, Masayuki; **Shimizu, Shoichi**
CORPORATE SOURCE: Fac. Bioresour., Mie Univ., Tsu, 514, Japan
SOURCE: Annals of the New York Academy of Sciences (1991), 646(Recomb. DNA Technol. I), 41-52
CODEN: ANYAA9; ISSN: 0077-8923
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A HindIII fragment of R. albus DNA encoding .beta.-glucosidase was cloned

into E. coli. The DNA sequence (3158 bp) was detd., and the longest potential encoding sequence consisted of 2841 bp (947 amino acids with the calcd. mol. wt. of 104,276. The deduced N-terminal amino acid sequence from the first (methionine) to the twentieth (glycine) was identical to that of the purified enzyme, suggesting that the gene for .beta.-glucosidase does not encode a signal peptide. The enzyme purified from the culture supernatant of the transformant had a mol. wt. of 120,000 and its max. activity was revealed at pH 6.5 and 30.degree.. **Reducing** reagents activated the enzyme, whereas the sulfhydryl group-blocking reagents and reaction products (glucose) inhibited the activity. Hydrolyzates of cellooligomers contained glucose as a major product, indicating that the enzyme acts as .beta.-glucosidase. The enzyme from the transformant revealed similar properties to that from R. albus, and both enzyme proteins were immunol. the same to each other, indicating that the cloned gene encodes .beta.-glucosidase from R. albus.

L41 ANSWER 15 OF 18 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1991:1657 HCAPLUS
DOCUMENT NUMBER: 114:1657
TITLE: Cloning of a cellobiose-transferring endo-1,4-.beta.-D-glucanase gene from Clostridium josui, its expression in Escherichia coli and properties of the purified translation product
AUTHOR(S): Ohmiya, Kunio; Fujino, Tsuchiyoshi; Sukhumavasi, Jiraporn; Sasaki, Takuji; **Shimizu, Shoichi**
CORPORATE SOURCE: Sch. Agric., Nagoya Univ., Nagoya, 464-01, Japan
SOURCE: Microbial Utilization of Renewable Resources (1989), 6, 384-94
CODEN: MURRE6
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The gene for a carboxymthylcellulose-degrading enzyme (cellulase) from C. josui was cloned in E. coli HB101 with pBR322. A 5.6-kb HindIII fragment encoding a cellulase was hybridized with chromosomal DNA of C. josui. The size of the cloned DNA fragment was **reduced** using PvuII, and the resulting active fragment with a size of 2 kb upon restriction with EcoRI and PstI was ligated into pUC118 at the SmaI sites (pUCJ1). The cellulase prodn. by E. coli in LB broth was enhanced approx. 3 times by controlling the pH at 6.5 and by **reducing** the concn. of NaCl to 80 mM. The translation product was purified by column chromatog. with DEAE-Bio Gel A, Sephacryl S-200HR, and Mono Q. The homogeneous protein revealed max. cellulase activity at pH 7.2-7.5 at 65-70 .degree.. The enzyme was very stable at temp. <45 .degree. (optimum growth temp. of C. josui) in the range of pH 4.5-9.0. The amino acid sequence of the enzyme from the N-terminus was Val-Glu-Glu-Asp-Ser-Ser-His-Leu-Ile-Thr-Asn-Gln-Ala-Lys-Lys-. The enzyme hydrolyzed cellotetraose to cellobiose and then transferred cellobiose to cellotetraose. The resulting cellohexaose was cleaved to cellotriose.

L41 ANSWER 16 OF 18 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1990:435515 HCAPLUS
DOCUMENT NUMBER: 113:35515
TITLE: Structure of a Ruminococcus albus endo-1,4-.beta.-glucanase gene
AUTHOR(S): Ohmiya, Kunio; Kajino, Tsutomu; Kato, Akemi; **Shimizu, Shoichi**
CORPORATE SOURCE: Sch. Agric., Nagoya Univ., Nagoya, 464-01, Japan
SOURCE: Journal of Bacteriology (1989), 171(12), 6771-5
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal

LANGUAGE: English
AB A chromosomal DNA fragment encoding an endo-1,4-.beta.-glucanase I (Eg I) gene from R. albus cloned and expressed in Escherichia coli with pUC18 was fully sequenced by the dideoxy-chain termination method. The sequence contained a consensus promoter sequence and a structural amino acid sequence. The initial 43 amino acids of the protein were deduced to be a signal sequence, since they are missing in the mature protein (Eg I). High homol. was found when the amino acid sequence of Eg I was compared with that of endoglucanase E from Clostridium thermocellum. Codon usage of the gene was not biased. These results suggested that the properties of the Eg I gene from R. albus were specified from the known .beta.-glucanase genes of the other organisms.

L41 ANSWER 17 OF 18 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1989:568616 HCAPLUS
DOCUMENT NUMBER: 111:168616
TITLE: Cloning of an endo-1,4-.beta.-D-glucanase gene from Clostridium josui and its expression in Escherichia coli
AUTHOR(S): Ohmiya, Kunio; Fujino, Tsuchiyoshi; Sukhumavasi, Jiraporn; **Shimizu, Shoichi**
CORPORATE SOURCE: Sch. Agric., Nagoya Univ., Nagoya, 464-01, Japan
SOURCE: Applied and Environmental Microbiology (1989), 55(9), 2399-402
CODEN: AEMIDF; ISSN: 0099-2240
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The gene for CM-cellulose-degrading enzyme (endoglucanase) from C. josui (FERM P-9684) was cloned in E. coli HB101 with pBR322. A 5.6-kilobase-pair HindIII fragment encoding an endoglucanase was hybridized with C. josui chromosomal DNA. The size of the cloned DNA fragment was **reduced** with PvuII, and the resulting active fragment (2 kilobase pairs, with restriction sites of EcoRI and PstI) was ligated into pUC118 at the SmaI sites (pUCJ1). The endoglucanase prodn. by E. coli JM103(pUCJ1) in Luria-Bertani broth was enhanced up to .apprx.3-fold by maintaining the pH at 6.5 and using 80 mM NaCl.

L41 ANSWER 18 OF 18 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1988:449552 HCAPLUS
DOCUMENT NUMBER: 109:49552
TITLE: Cloning of the cullulase gene from Ruminococcus albus and its expression in Escherichia coli
AUTHOR(S): Ohmiya, Kunio; Nagashima, Kyo; Kajino, Tsutomu; Goto, Etsuo; Tsukada, Akiko; **Shimizu, Shoichi**
CORPORATE SOURCE: Sch. Agric., Nagoya Univ., Nagoya, 464-01, Japan
SOURCE: Applied and Environmental Microbiology (1988), 54(6), 1511-15
CODEN: AEMIDF; ISSN: 0099-2240
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The gene for cellulase from R. albus F-40 was cloned in Escherichia coli HB101 with pBR322. A 3.4-kilobase-pair HindIII fragment encoding cellulase hybridized with the chromosomal DNA of R. albus. The Ouchterlony double-fusion test gave a single pptn. line between the cloned enzyme and the cellulase from R. albus. The size of the cloned fragment was **reduced** by using HindIII and EcoRI. The resulting active fragment had a size of 1.9 kilobase pairs; and the restriction sites for EcoRI, BamHI, PvuII, EcoRI, PvuII, and HindIII, in that order, were ligated into pUC19 at the EcoRI and HindIII sites (pURA1). Cellulase prodn. by E. coli JM103(pURA1) in Luria-Bertani broth was remarkably

enhanced .ltoreq.80-fold by controlling the pH and by **reducing** the concn. of NaCl in the broth to 80 mM.